ACKNOWLEDGMENT

Applicants acknowledge the election made in Paper No. 10 of claims 1-4 in response to the earlier restriction requirement. Claims 1-4 have been elected and are pending in this application. The sequence listing was entered and the informalities in the drawings are noted. Suitable corrections to the drawings will be made within the three-month shortened statutory period following receipt of any "Notice of Allowabilty." Priority to provisional application SN 60/166,663, filed 11/19/1999 is acknowledged.

SEQUENCE LISTINGS AND TRADE NAMES

The specification is amended to insert Sequence ID numbers for sequence listings longer than 10. The formalities for the recitation of trademarks are also corrected. A corrected sequence listing is also enclosed. Pursuant to 37 C.F.R. § 1.825, the computer-readable and paper form are the same and include no new matter.

WRITTEN DESCRIPTION UNDER 35 U.S.C. § 112

The amendment to claim 1 traverses the pending written description issue.

DEFINITENESS UNDER 35 U.S.C. § 112

The amendment to the pending claims traverses the rejections set out at paragraph 10, subparagraphs a-d of the pending action. Specifically, the phrase "comprising an isolated" is substituted for the phrase "comprising isolated," and the term "als1" is recited in full. The description of the als1 protein in the vaccine of the present invention is also amended in the pending claims and this amendment overcomes the issues raised in subparagraphs a and b.

THE AMENDED CLAIMS ARE NOT ANTICIPATED BY THE HOYER ET AL. (J.Bacteriol. 1998) REFERENCE.

The amended claims are clarified to specify that the composition of the invention is a "pharmaceutical composition" that is useful as a vaccine to promote an immune response against the als1P protein. There is no meaningful disclosure in the Hoyer et al. reference of the formulation of an als1P protein to yield an effective result as a vaccine. The amended claim language distinguishes the Hoyer et al. reference by requiring that the pharmaceutical composition of the claim have the operative property of yielding a protective immune response.

THE CLAIMS, AS AMENDED, ARE NOT RENDERED UNPATENTABLE BY 35 U.S.C. § 103.

The nature of Applicants' invention is based on the discovery that the als1P protein, a known molecule, can be formulated as an effective vaccine to provide protection against infection caused by the *candida* species, particularly *candida albicans*. Hoyer et al., as the Examiner notes, do not disclose any useful immunological function for the als1P protein and does not make the leap from speculating that the als1P protein has a "potential role" in adhesin. The entire statement made by Hoyer et al. (1995) is:

> Possible functions for ALS1
>
> The AGα1 gene and corresponding protein have been extensively characterized (Lipke et al., 1989; Wojciechowicz et al., 1993). The AGα1 protein is known to mediate cell – cell adhesion in *S. cerevisiae* during mating of haploid cells (Lipke et al., 1989). The existence of a sample agglutination assay has enabled researchers to determine that the binding domain of AGα1 is located between residues 278 and 350 (Wojciechowicz et al., 1993). Because *C. albicans* has never been observed to undergo meiosis (reviewed by Odds, 1988), it is unlikely that ALS1 functions in *C. albicans* to facilitate meting. Since agglutinins in yeast species function as adhesion molecules (reviewed by Lipke and Kurjan, 1992), a potential role can be envisioned for ALS1 in other aspects of cell

> adhesion, particularly adhesion of this fungal pathogen to host cells, a property which has been demonstrated to contribute to the virulence of *C. albicans* (reviewed by Calderone and Braun, 1991). While the nature of ALS1 function remains to be determined, such experiments are not straightforward because there are many possible interactions that are likely to be media dependent. (emphasis added).

From this passage of the Hoyer et al. article, the office may not conclude that the composition inherently possesses the same function as the claimed vaccine in the instant application, because the reference does not disclose a formulation that has been demonstrated to yield any immune response, nor, in particular, the protective immune response of the vaccine recited in the claims. Hoyer et al. explicitly state: "The nature of ALS1 is yet to be determined..." Because the content of the pending claims is nowhere found in Hoyer et al., or anywhere in any of the cited references, Applicants have disclosed novel and nonobvious subject matter.

The leap from a disclosure of a molecule with a "potential role" in adhesion to a viable vaccine is significant. Hoyer et al. does not disclose that the molecule actually mediates adhesion, that it can be targeted by immunotherapy, or that an immune response against such a molecule would protect against the virulence of the organism. Under such circumstances, § 103 cannot be applied without an impermissible modification of the teachings of Hoyer et al.

Two well-established principles of the law of obviousness under 35 U.S.C. § 103 dictate that first, the cited prior art references must be considered "as a whole" and must suggest the desirability and thus the obviousness of making the invention. The Examiner proposes that one of ordinary skill in the art could have reformulated the ALS1 molecule to arrive at the claimed vaccine, however, this rationale cannot be used to render a claimed composition obvious unless the suggestion or rationale for the modification was recognized in the art. As such, there is no basis in Hoyer et al. to go

looking for a vaccine. An obviousness rejection cannot be based on the proposition that one of ordinary skill in the art would have been motivated to engage in an open-ended exercise to try all manner of experiments until one possible variation reaches an applicant's claimed invention. Such an exercise is merely an extension of the forbidden "obvious to try" standard, See In re O'Farrell, 7 USPQ2d 1673 (Fed. Cir. 1988).

Furthermore, such a reformulation would violate the rule explained in In re Gordon, 733 F.2d 900 (Fed. Cir. 1984) wherein the Federal Circuit held that a prior art reference may not be modified in a way that would render the prior art invention unsatisfactory for its intended purpose (See MPEP § 2143.01).

CLAIM OBJECTIONS

The objections to claims 1 and 4 have been overcome by the above amendments to the claims.

Respectfully submitted,

ORRICK, HERRINGTON & SUTCLIFFE LLP

Dated: April 9, 2003

By: _____
Kurt T. Mulville
Reg. No. 37,194

4 Park Plaza, Suite 1600
Irvine, CA 92614
949/567-6700 Telephone
949/567-6710 Facsimile

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IN THE SPECIFICATION:

On page 10, line 6, please replace with the following:

Figure 7 is the polynucleotide (SEQ ID NO.7) polypeptide sequence of Als1p (SEQ ID NO.8).

On page 11, line 14 through page 12, line 3, please replace with the following:

The URA blaster technique was used to construct a null mutant of C *albicans* that lacks expression of the Als1p. The *als1/als1* mutant was constructed in *C. albicans* strain CAI4 using a modification of the Ura-blaster methodology [W. A. Fonzi and M. Y. Irwin, *Genetics* **134**, 717 (1993)] as follows: Two separate *als1-hisG-IRA3-hisG-als1* constructs were utilized to disrupt the two different alleles of the gene. A 4.9 kb *ALS1* coding sequence was generated with high fidelity PCR (Boehringer Mannheim, Indianapolis, IN) using the primers: 5'-CCGCTCGAGATGCTTCAACAATTTACATTGTTA-3' (SEQ ID NO.1) and 5'-CCGCTCGAGTCACTAAATGAACAAGGACAATA3' (SEQ ID NO.2). Next, the PCR fragment was cloned into pGEM-T vector (Promega, Madison, WI), thus obtaining pGEM-T-*ALS1*. The *hisG-URA3-hisG* construct was released from pMG-7 by digestion with *KpnI* and *Hind3* and used to replace the portion of *ALS1* released by *KpnI* and *Hind3* digestion of pGEM-T-*ALS1*. The final *als1-hisG-URA3-hisG-als1* construct was released from the plasmid by digestion with *XhoI* and used to disrupt the first allele of *ALS1* by transformation of strain CAI-4.

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On page 13, line 5 through page 14, line 17, please replace with the following:

Referring to Figure 1, a comparison of the ALS1/ALS1 and als1/als1 strain showed that the ALS1 null mutant was 35% less adherent to endothelial cells than *C. albicans* CAI-12. To reduce background adherence, the adherence of the wild-type strain grown under non-ALS1 expressing conditions was compared with a mutant autonomously expressing A1s1p. This mutant was constructed by integrating a third copy of ALS1 under the control of the constitutive ADH1 promoter into the wild-type *C.* albicans. To achieve constitutive expression of the ALS1 in *C. albicans,* a blunt-ended PCR generated *URA3* gene is ligated into a blunt-edged Bg12 site of pOCUS-2 vector (Novagen, Madison, WI), yielding pOU-2. A 2.4 kb *Not1-Stul* fragment, which contained *C. albicans* alcohol dehydrogenase gene (ADH1) promoter and terminator (isolated from pLH-ADHpt, and kindly provided by A. Brown, Aberdeen, UK), was cloned into pOU-2 after digestion with *Not1* and *Stul.* The new plasmid, named pOAU-3 had only one *Bg12* site between the *ADH1* promoter and terminator. *ALS1* coding sequence flanked by *BamH1* restriction enzyme sites was generated by high fidelity PCR using pYF-5 as a template and the following primers: 5'-CGGGATCCAGATGCTTCA-ACAATTTACATTG-3' (SEQ ID NO.3) and 5'-CGGGATCCTCACTAAATGAACAAGGACAATA-3' (SEQ ID NO.4). This PCR fragment was digested with *BamH1* and then cloned into the compatible *Bg12* site of pOAU-3 to generate pAU-1. Finally, pAU-1 was linearized by *Xbal* prior to transforming *C. albicans* CAI-4. The site-directed integration was confirmed by Southern Blot analysis. Referring to Figure 1B, overexpressing *ALS1* in this P_{ADH1}-*ALS1* strain resulted in a 76% increase in adherence to endothelial cells, compared to

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the wild-type *C. albicans*. In comparing endothelial cell adherence of the wild-type to that of the overexpressing mutant, yeast cells were grown overnight in YPD at 25°C (non-inducing condition of Als1p). Als1p expression was not induced to reduce the background adherence of the wile-type, thus magnifying the role of Als1p in adherence through P_{ADH1}-*ALS1* hybrid gene. The adherence assay was carried out as described above. Statistical treatment was obtained by Wilcoxon rank sum test and corrected for multiple comparisons with the Bonferroni correction. $P<0.001$.

A monoclonal anti-Als1p murine IgG antibody was raised against a purified and truncated N-terminus of Als1p (amino acid #17 to #432) expressed using CLONTECH™ [Clontech] YEXpress (™) yeast expression system (Palo Alto, CA). The adherence blocking capability of these monoclonal anti-Als1p antibodies was assessed by incubating *C. albicans* cells with either anti-Als1 antibodies or mouse IgG (Sigma, St. Louis, MO) at a 1:50 dilution. After which the yeast cells were used in the adherence assay as described above. Statistical treatment was obtained by Wilcoxon rank sum test and corrected for multiple comparisons with the Bonferroni correction. $P<0.001$. The results revealed that the adherence of the P_{ADH1}-ALS1 strain was reduced from 26.8%$\pm$ 3.5% to 14.7%$\pm$ 5.3%. Thus, the effects of ALS1 deletion and overexpression demonstrate that Als1p mediates adherence of *C. albicans* to endothelial cells.

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On page 16, lines 5 - 14, please replace with the following:

If Efg1p stimulates the expression of *ALS1*, which in turn induces filamentation, the expression of *ALS1* in the *efg1/efg1* strain should restore filamentation. A functional allele of *ALS1* under the control of the *ADH1* promoter was integrated into the *efg1/efg1* strain. To investigate the possibility that *ALS1* gene product might complement the filamentation defect in *efg1* null mutant, an Ura *efg1* null mutant was transformed with linearized pAU-1. Ura^+ clones were selected and integration of the third copy of *ALS1* was confirmed with PCR using the primers: 5'-CCGTTTATACCATCCAAATC-3' (SEQ ID NO.5) and 5'-CTACATCCTCCAATGATATAAC-3' (SEQ ID NO.6). The resulting strain expressed *ALS1* autonomously and regained the ability to filament on Lee's agar. See Figures 4B and C. Therefore, Efg1p induces filamentation through activation of *ALS1* expression.

On page 18, line 20 through page 19, line 7, please replace with the following:

The fragment of *ALS1* was ligated into pQE32 to produce pINS5. In this plasmid, the protein is expressed under control of the *lac* promoter and it has a 6-hits tag fused to its N-terminus so that it can be affinity purified. We transformed *E. coli* with pINS5, grew it under inducing conditions (in the presence of IPTG), and then lysed the cells. The cell lysate was passed through a Ni^{2+} -agarose column to affinity purify the *ALS1*-6His fusion protein. This procedure yielded substantial amounts of *ALS1*-6His. The fusion protein was further purified by SDS-PAGE. The band containing the protein was excised from the gel so that polyclonal rabbit antiserum can be raised against it. It will be appreciated by one skilled in the art that the surface adhesin protein according to the invention may be prepared and purified by a variety of known processes without departing from the spirit of the present invention. The underlying polynucleotide sequence and the polypeptide sequence of Als1p are [is] listed in Figure 7 (SEQ ID NOS.7 and 8).

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IN THE CLAIMS:

Please amend claims 1 and 3 and cancel claims 2 and 4. Please add new claim 9.

1. A vaccine comprising:

a pharmaceutical composition comprising an isolated and purified agluttin like sequence (als1) cell surface adhesin protein obtained from [a] *Candida* [strain] *albicans,* wherein the vaccine produces an effective immune response in a patient to block adhesin of *Candida albicans* to endothelial cells.

3. The vaccine of Claim 1 [2], wherein the protein is an [a fragment encompassing the N-terminus of Als1p and] N-terminal fragment and contains[ing] the adhesin binding site.

9. The vaccine of claim 1 wherein the protein is SEQ ID NO.7.